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Original article

Anti-HBc prevalence among Croatian blood donors in a 14-year period (2004–2017): Assessment of trends, risks and need for implementing routine testing

Prévalence anti-HBc chez les donneurs de sang croates sur une période de 14 ans (2004–2017) : tendances, infectiosité, riques résiduels, OBIs et nécessité du dépistage

Manuela Miletić[a,*], Jasna Bingulac-Popović[a], Miljana Stojić Vidović[a], Ana Hećimović[a], Mirka Berendika[b], Ivana Babić[a], Vesna Đogić[a], Marko Samardžija[c], Karmela Barišić[d], Irena Jukić[a], Ivanka Mihaljević[a]

[a] *Croatian Institute of Transfusion Medicine (CITM), Petrova 3, 10000 Zagreb, Croatia*
[b] *Abbott Diagnostic Croatia, Koranska 2, 10000 Zagreb, Croatia*
[c] *Clinical Hospital Osijek, Huttlerova 4, 31000 Osijek, Croatia*
[d] *Faculty of Pharmacy and Biochemistry, University of Zagreb, Ante Kovačića 1, 10000 Zagreb, Croatia*

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ABSTRACT

Objectives. – The anti-HBc prevalence over a 14-years period (2004–2017), trends, infectivity, residual risk, and need for testing in blood donors (BD) of the Croatian Institute of Transfusion Medicine were assessed.
Material and methods. – Anti-HBc was tested in 19,969 BD serum samples collected in 2004 ($N = 7561$), 2013 ($N = 7318$) and 2017 ($N = 5090$). All serums were initially screened for HBsAg, anti-HCV, HIV Ag/Ab, and anti-TP. 2013 and 2017 samples were also tested by ID-NAT.
Results. – Over a 14-years period, the anti-HBc prevalence significantly decreased among Croatian BD (5.24% in 2004, 2.56% in 2013, and 1.32% in 2017). Similarly, the prevalence of anti-HBc-only profiles decreased from 0.62% in 2004, 0.25% in 2013, and 0.21% in 2017. The 4-time decreasing trend was observed in all age groups of BD from 2017 but mostly among repeat donors (5.90% to 1.38%). First-time donors showed no significant difference in anti-HBc prevalence probably due to their younger age (< 29 years) and HBV vaccine status. However, similar anti-HBs carriage rates (80.56%, 87.57%, and 82.09%) were reported in anti-HBc positive donors over the study period. HBsAg and HBV DNA were not detected. No OBI infection was found in the study despite an OBI frequency of 1:10,900 donations previously reported in Croatia. A HBV decreasing residual risks of 68, 88, and 12 per million donations were estimated for years 2004, 2013, and 2017, respectively.
Conclusion. – Anti-HBc testing is an additional measure of preventing HBV infection by transfusion. Implementation of anti-HBc testing will result in the deferral of 1.3% BD and should be supported by cost-benefit analyses.

Mots clés :
Donneurs de sang
Séroprévalence anti-HBc
OBI

RÉSUMÉ

Buts/Objectifs. – La prévalence anti-HBc, ses tendances évolutives dans le temps, l'infectiosité, le risque résiduel infectieux et la nécessité du dépistage ont été évalués sur une période de 14 ans (2004–2017) chez les donneurs de sang (DS) de l'Institut croate de medecine transfusionnelle.

* Corresponding author: Croatian Institute of Transfusion Medicine (CITM), Petrova 3, 10000 Zagreb, Croatia.
E-mail addresses: manuela.miletic@hztm.hr (M. Miletić), jasna.bingulac-popovic@hztm.hr (J. Bingulac-Popović), miljana.stojic.vidovic@hztm.hr (M. Stojić Vidović), ana.hecimovic@hztm.hr (A. Hećimović), mirka.berendika@abbott.com (M. Berendika), ivana.babic@hztm.hr (I. Babić), vesna.dogic@hztm.hr (V. Đogić), drmarkosamardzija@gmail.com (M. Samardžija), karmela.barisic@pharma.hr (K. Barišić), irena.jukic@hztm.hr (I. Jukić), imihalj263@gmail.com (I. Mihaljević).

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Matériels et méthodes. – Un nombre total de 19 969 échantillons de sérum de DS (7561, 7318 et 5090 collectés en 2004, 2013 et 2017, respectivement) ont été testés pour l'anti-HBc après dépistage obligatoire de l'AgHBs, des anti-VHC, des Ag/Ac VIH et des anti-TP en 2004, et introduction du dépistage des génomes viraux en dons individuels (ID-NAT).
Résultats. – Sur une période de 14 ans, la prévalence des anti-HBc a significativement diminué chez les DS croates (5,24 % en 2004, 2,56 % en 2013 et 1,32 % en 2017). La prévalence du profil anti-HBc isolé a diminué de façon similaire, passant de 0,62 % en 2004 à 0,25 % en 2013 et à 0,21 % en 2017. La tendance à la baisse d'un facteur 4 a été observée dans tous les groupes d'âge de DS en 2017, mais principalement chez les donneurs réguliers (de 5,90 % à 1,38 %). Aucune différence significative de la prévalence anti-HBc n'a été observée chez les nouveaux donneurs, probablement en raison de leur plus jeune âge (< 29 ans) et de leur statut vaccinal. Cependant, des prévalences anti-HBs similaires (80,56 %, 87,57 % et 82,09 %) ont été observées chez les donneurs anti-HBc positifs au cours de la période d'étude. L'AgHBs et l'ADN VHB n'ont pas été détectés, excluant toute infection OBI malgré une fréquence de ce type d'infection de 1:10 900 dons précédemment décrite en Croatie. Des risques infectieux VHB résiduels de 68, 88 et 12 par million de dons ont été respectivement estimé pour les années 2004, 2013 et 2017.
Conclusion. – Le dépistage anti-HBc est une mesure supplémentaire de prévention de la transmission par transfusion de l'infection VHB. La mise en œuvre du dépistage anti-HBc entraînera l'exclusion supplémentaire de 1,3 % des donneurs et doit être étayée par des analyses de coût-efficacité.

1. Introduction

The risk of transfusion-transmitted hepatitis B virus (HBV) infection is very low but remains higher than the transmission risk associated with other viruses for which mandatory testing is also in place [1]. This difference in transfusion-transmission risk may be related to the variability of the clinical presentation of HBV infection and the associated host's immune responses, the viral replication and serological markers dynamics that makes both the selection of blood donors and testing more difficult. Which tests are to be used for blood donor (BD) screening will depend on the HBV prevalence in the general and BD populations, the ratio of first-time (FTD) and repeat donors (RD), self-sufficiency in blood supply, and the country's economic resources.

According to the WHO report [2], several methods for HBV BD testing are available: a) HBsAg testing is the most common strategy globally, used by 56% (98/176) of WHO member states (Europe: 32.5% or 14/43); b) HBsAg and anti-HBc testing is used by 17.6% (31/176) of countries worldwide (Europe: 9.3% or 4/43) including 5 countries (3 of which are EU member states) that implemented selective anti-HBc tests; c) HBV DNA and HBsAg testing is used by 11.9% (21/176) of countries (Europe: 27% or 12/43); and d) HBV DNA, HBsAg and anti-HBc testing is used in 7.4% (12/176) of countries including 11.6% (5/43) of European countries. Additional 8 countries (including 5 EU member states) implemented the 3-test strategy with selective anti-HBc testing.

The implementation of molecular testing allowed the detection of occult hepatitis B virus infection (OBI) also characterized by the presence of detectable anti-HBc [3,4]. Anti-HBc testing proved to be more efficient than NAT when HBV DNA load corresponds to the lower limit of sensitivity of the NAT assay (< 3 IU/mL HBV DNA). However, in the absence of molecular testing, qualitative anti-HBc testing does not discriminate between potentially infectious and non-infectious samples [5–8]. Anti-HBc was used recently as a supplementary test in the HBV DNA ID-NAT confirmation algorithm for initially reactive (IR) and non-repeated reactive (NRR) blood donors with HBV DNA load usually below 10 IU/mL and not confirmed by using quantitative highly sensitive tests or discriminatory ID-NAT tests.

Croatian Institute of Transfusion Medicine (CITM) is a national reference centre for transfusion medicine which collects more than 50% of all blood donations in Croatia every year (approx. 100,000 donations). The need to change BD HBV testing strategy in Croatia by introducing anti-HBc was assessed first in 2005 by using archive samples of BDs collected in 2004. This study aimed to establish the frequency of anti-HBc-only positive donors and their infectivity as HBV DNA testing was limited to this group of donors [9]. Two other studies investigating the efficiency of the anti-HBc marker in BD testing were carried out in 2013 and 2017. The objectives of the present study were to document anti-HBc prevalence in Croatian BDs over a 14-years period, analyse trends in anti-HBc prevalence according to age and gender and according to number of donations, to evaluate the potential infectivity of anti-HBc positive BDs by testing for HBV DNA presence. Data collected will provide elements to evaluate the impact of a possible introduction of anti-HBc testing of blood donors on HBV residual risk and potential loss of blood donors.

Table 1
Blood donor characteristics in the three studies and residual risk calculation.

Characteristics of blood donations and donors	Year of study		
	2004	2013	2017
Total N blood donations	71,897	100,920	105,323
FTD %	10.1	6.1	6,4
RD %	89.9	93.9	93.6
Total N donors	39,011	49,003	50,445
FTD %	18.6	12.6	13.4
RD %	81.4	87.4	86.6
Female (F) donors %	20.4	21	22.4
HBV prevalence/10^5 FTD	220	97	29.6
HBV incidence/10^5 RD	12.6	28	9.2
Anti-HBc tested	7561	7318	5090
F donors %	17.4	13.1	16
M donors %	82.6	86.9	84
Residual risk (RR)[a]	68	88	12
HBV Look-back/Trace back	4/0	11/0	4/0

FTD; first time donors; RD; repeat donors.
[a] Calculation of RR by European Medicines Agency 2016.

2. Material and methods

2.1. Blood samples

Archived residual serum samples from blood donations collected in October 2004 and in July–August 2013 and prospectively collected residual samples in May 2017 by CITM were included in the study. Samples were collected from consecutive donations and

Table 2
HBV tests used for blood donor testing in the three studies.

Test	Year of study		
	2004	2013	2017
Anti-HBc screening test	Hepatitis B Virus Core Antigen ORTHO HBc ELISA Test System	Architect Anti-HBc II (Abbott)	Prism HBcore (Abbott)
Anti-HBc alternative test 1	ETI-AB-COREK PLUS (DiaSorin)	Monolisa Anti-HBc PLUS (Bio-Rad)	Architect Anti-HBc II (Abbott)
Anti-HBc alternative test 2	Vitros aHBc (Ortho)	Vidas Anti-HBc Total II (BioMerieux)	Vidas Anti-HBc Total II (BioMerieux)
HBsAg screening test	Enzygnost HBsAg 5.0 EIA (Dade-Behring)	Prism HBsAg (Abbott)	Prism HBsAg (Abbott)
HBsAg alternative test	Murex HBsAg Version 3	Monolisa HBsAg ULTRA (Bio-Rad)	Monolisa HBsAg ULTRA (Bio-Rad)
Anti-HBs	Vitros (Ortho)	Architect (Abbott)	Architect (Abbott)
Anti-HBc IgM	Vitros (Ortho)	Architect (Abbott)	Architect (Abbott)
Anti-HBe	Vitros (Ortho)	Architect (Abbott)	Not done
HBeAg	Vitros (Ortho)	Architect (Abbott)	Not done
HBV DNA test	HBV MONITOR/Cobas Amplicor (Roche)	ID-NAT Procleix Ultrio Plus (HBV/HCV/HIV) (Grifols)	ID-NAT Procleix Ultrio Elite (HBV/HCV/HIV-1/HIV-2) (Grifols)
	HPS/HBV Cobas TaqMan48 (Roche)		

therefore were not stratified according to gender, age and the number of donations. BDs were classified in the following age groups: <20, 20–24, 25–29, 30–34, 35–39, 40–44, 45–49, 50–54, 55–59 and ≥60. Basic characteristics of the tested BD population in the 2004, 2013 and 2017 studies of the CITM are shown in Table 1. Serum samples were prepared for testing according to the standard operating procedures, and testing was done according to the manufacturer's instructions. The study was performed with the consent of the competent Ethical Committee pursuant to the Declaration of Helsinki.

2.2. Serological and molecular screening

Table 2 shows assays used for serological markers and HBV DNA testing according to the manufacturers' instructions.

2.3. Confirmation algorithms for anti-HBc reactivity

All donors tested anti-HBc positive in 2004, 2013 and 2017 were retested with the same anti-HBc assay and two alternative assays (Table 2). Repeatedly reactive (RR) samples confirmed with at least two anti-HBc assays were considered confirmed positive. All anti-HBc positive samples collected in 2004 and 2013 were tested for all HBV markers, whereas all anti-HBc positive samples from 2017 were tested only for anti-HBc IgM and anti-HBs. In all samples, HBsAg was also tested with an alternative test to exclude possible "s" mutations in the HBV genome. HBV DNA was tested in anti-HBc positive samples with a quantitative HBV DNA test (HBV Monitor/Cobas Amplicor, sensitivity of 54 IU/mL, and HPS/HBV Cobas TaqMan 48, sensitivity of 6 IU/mL) in 2004, and with ID-NAT assays in 2013 (Procleix Ultrio Plus test, 95% LOD for HBV 3.4 IU/mL) and 2017 (Procleix Ultrio Elite test, 95% LOD for HBV 4.3 IU/mL).

2.4. Statistical methods

The MedCalc v16.2.1 program was used for statistical analysis of results. A test of proportion (Chi^2 test) with the 0.05 significance level was used for establishing the difference in the number of subjects/BDs according to various categories, anti-HBc prevalence, presence of HBV DNA and positivity for other HBV markers. The Robert G. Newcombe's method was used for calculating 95% of confidence intervals.

3. Results

3.1. Anti-HBc prevalence

Table 3 shows the anti-HBc prevalence in blood donors in the years of study. A statistically significant decrease in anti-HBc prevalence was observed in 2013 as compared with 2004 (from 5.24% to 2.56%; $\chi^2 = 75.741$; $P < 0.001$), as well as in 2017 as compared with 2013 (from 2.56% to 1.32%; $\chi^2 = 22.97$; $P < 0.05$). The difference in anti-HBc prevalence in 2013 resulted from a 5.90% to 2.64% decreased prevalence in repeat donors (RD) ($\chi^2 = 87.670$; $P < 0.001$), whereas the decrease in anti-HBc prevalence in 2017 as compared with 2013 is a result of decreased prevalence in RDs from 2.64% to 1.38% ($\chi^2 = 4.04$; $P < 0.05$) and in first time donors (FTD) from 0.88% to 0.32% ($\chi^2 = 1.669$; $P < 0.05$).

Fig. 1 shows the anti-HBc prevalence reduction index in BDs, both overall and according to gender. Anti-HBc prevalence has reduced by 4 times in the 14-years period (4.11 in male donors and 3.09 in female donors). Interestingly enough, the 2013/2017 prevalence reduction index of 1.94 has almost reached the reduction index of 2.05 achieved in the 9-years period between 2004 and 2013. The greatest prevalence reduction was recorded in age groups 25–40 (10–18 times).

3.2. Anti-HBc prevalence according to donors' age and gender

The greatest anti-HBc prevalence reduction in male BDs in 2013 was registered in the age groups 50–60, whereas a statistically significant reduction was registered in groups older than 30 years of age ($P < 0.05$). A statistically significant prevalence reduction ($P < 0.05$) in 2017 compared with 2013 was observed in all groups older than 20 years of age.

Anti-HBc prevalence reduction in female BDs in 2013 compared with 2004 was observed only in the age group 40–44 ($P = 0.03$), and the reduction is not statistically significant in 2017 as compared with 2013 ($\chi^2 = 1.24$; $P = 0.265$), except in the age groups of 25–34, 40–44 and 50–59 years ($P < 0.05$).

3.3. Anti-HBc prevalence in FTD and RD

Anti-HBc prevalence in the population of FTDs in 2013 (0.88%) as compared with 2004 (1.37%), as well as the prevalence in 2013 as compared with 2017 (0.32%) does not indicate a statistically significant difference ($\chi^2 = 0.493$; $P = 0.492$)/($\chi^2 = 0.833$; $P = 0.361$). A significant anti-HBc prevalence reduction in the population of RDs was registered in 2013 as compared with 2004, in all age groups ($\chi^2 = 88.457$; $P < 0.01$) with the exception of the age group 20–24 ($\chi^2 = 1.217$, $P = 0.27$). A reduction in prevalence in 2017 as compared with 2013 was registered in all age groups ($\chi^2 = 243.74$; $P < 0.05$).

3.4. Anti-HBc-only prevalence

Anti-HBc-only prevalence in donors tested in 2004 was 0.62%, whereas in 2013 it was 0.25%, which showed a statistically significant difference ($\chi^2 = 11.65$, $P < 0.01$). Anti-HBc-only prevalence in donors tested in 2017 was 0.21%, implying a continuous decline

Table 3
Anti-HBc prevalence in blood donors in the years of study.

Anti-HBc prevalence % (95%CI) in the different groups of tested donors							
Year	Anti-HBc Anti-HBc-only	FTD		RD		F	M
2004	5.24 (4.76–5.77)	1.37 (0.83–2.25)		5.90 (5.35–6.50)			
	0.62 (0.47–0.82)	F 1.43 (0.61–3.30)	M 1.34 (0,73–2.45)	F 5.72 (4.42–7.37)	M 5.93 (5.34–6.59)	4.57 (3.57–5.84)	5.38 (4.85–5.97)
2013	2.56 (2.22–2.95)	0.88 (0.30–2.56)		2.64 (2.29–3.04)		2.20 (1.44–3.34)	2.61 (2.05–3.03)
	0.25 (0.16–0.39)	F 0.0 (0.00–2.56)	M 1.55 (0.53–4.45)	F 2.59 (1.70–3.93)	M 2.64 (2.27–3.07)		
2017	1.32 (1.04–1.67)	0.32 (0.06–1.80)		1.38 (1.09–1.75)		1.48 (0.85–2.57)	1.31 (1.01–1.70)
	0.21 (0.12–0.39)	F 0.0 (0.00–3.15)	M 0.52 (0.09–2.89)	F 1.59 (0.89–2.82)	M 1.35 (1.04–1.75)		

FTD: first time donors; RD: repeat donors; F: female donors; M: male donors.

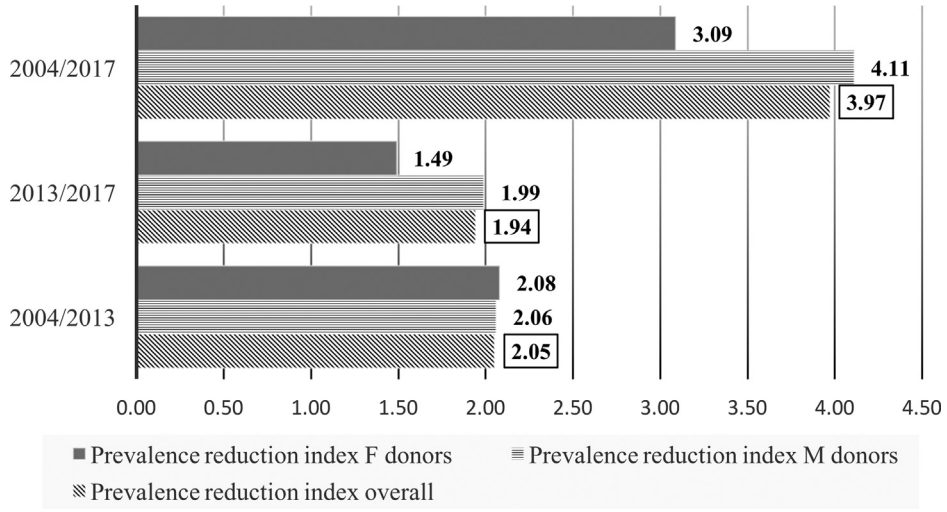

Fig. 1. Anti-HBc prevalence reduction index in BDs, both overall and according to gender. F: female donors; M: male donors.

Table 4
Results of other serological and molecular tests conducted on samples of anti-HBc tested BDs.

Test	Year of testing								
	2004			2013			2017		
	n tested	*n* positive	%	*n* tested	*n* positive	%	*n* tested	*n* positive	%
HBsAg routine and alternative test	7561	0	0	7318	0	0	5090	0	0
Anti-HBc	7561	396	5.24	7318	187	2.56	5090	67	1.32
Anti-HBc IgM	396	1(gz[a])	0.25	187	0	0	67	0	0
Anti-HBs	396	319	80.56	185	162	87.57	67	55	82.09
Anti-HBe	396	147	37.12	187	62	33.16	0	nt[b]	–
HBeAg	396	0	0	187	0	0	0	nt[b]	–
HBV DNA	47	0	0	7318	0	0	5090	0	0

[a] Grey-zone.
[b] Not tested.

trend; however, it is not significantly different as compared with 2013 ($\chi^2 = 0.206$, $P < 0.650$). Shares of anti-HBc-only positives among anti-HBc positives amounted to 11.8%, 9.6% and 16.4%, and the differences are not statistically significant ($\chi^2 = 0.46$, $P = 0.497$, $\chi^2 = 2.25$, $P = 0.134$). Statistically significant difference was found in the frequency of anti-HBc-only positives among anti-HBc positives in blood donors younger than 30 years, resulting in a significant prevalence reduction (23% in 2004/0% in 2013 – $\chi^2 = 50.88$, $P < 0.001$, and 0% in 2017 – $\chi^2 = 19{,}14$, $P < 0.001$).

3.5. Presence of other HBV markers

Table 4 shows test results of other HBV markers and HBV DNA. HBsAg was not confirmed in groups tested with two different HBsAg tests. Testing results have identified almost the same frequency of anti-HBs (80.56% and 87.57%) and anti-HBe (37.12% and 33.16%) positives among anti-HBc positive BD in 2004 and 2013 ($\chi^2 = 3.12$; $P = 0.077$) ($\chi^2 = 0.29$; $P = 0.587$). No statistically significant difference was observed in anti-HBs positive rates among

anti-HBc positive BDs from 2013 (87.57%) and 2017 (82.09%) ($\chi^2 = 1.03$; $P = 0.310$). Anti-HBs titer greater than 100 IU/L was registered in 54.1%, 55.1% and 52.3% of anti-HBc positives in the three groups with a statistically significant difference for all three years ($P < 0.0001$). Anti-HBs titer greater than 200 IU/L was registered in 47.5%, 43.2% and 40.4% of anti-HBc positive from 2004, 2013 and 2017 (statistically significant difference; $P < 0.0001$).

3.6. HBV DNA tests in BD samples tested for anti-HBc

HBV DNA was tested in 47 anti-HBc-only positive BDs from 2004 and all samples tested negative. Similarly, no viral DNA was detected by ID-NAT in 7,318 and 5,090 BDs from 2013 and 2017, respectively (Table 4).

4. Discussion

The results showed a significant reduction of anti-HBc prevalence from 5.24% to 1.32% over the 14-years period, mostly as a result of reduced prevalence in repeat blood donors (5.90% vs. 1.38%). The reduction in anti-HBc prevalence between 2004 and 2013 was not associated with gender (reduction index: repeat female BDs (F RDs)/reduction index: repeat male BD (M RDs) = 2.21/2.24), whereas prevalence reduction in male repeat donors was greater in 2017 compared to 2013 (M RDs/F RDs reduction index = 1.96/1.64). The greatest anti-HBc prevalence reduction in male repeat donors was observed in the 25–40 age group, and in female donors in the 40–49 age group. Possible general factors responsible for this anti-HBc prevalence reduction, apart from the RD's age shift, may be related to prevalence reduction in the general population due to the efficiency of preventive measures such as mandatory testing of pregnant women, universal vaccinations, safety levels in health-care and paramedic institutions, as well as of transfusion services, based on selection and testing of BDs, supported by national IT system and unique BD's database and centralised confirmatory testing. The important specific factor for reducing anti-HBc yield is certainly implementation of ID-NAT BD's testing in Croatia in 2013. Therefore, if the prevalence of anti-HBc in 2017 (1.32%) is corrected for OBI BDs in CITM (total $N = 27$, 2013 till 2017), anti-HBc prevalence would be 1.85%.

Anti-HBc prevalence decrease in FTD (1.37% in 2004, 0.88% in 2013, and 0.32% in 2017) was not significant. Blood donors from 2004 were only sporadically vaccinated against HBV whereas the rate of vaccinated donors increased to 10.4% in 2013 and 27.3% in 2017 (donors younger than 25 and 30 years of age, respectively). Anti-HBc prevalence in donors younger than 25 years of age was 0.32% in 2013 (FTD 0%, RD 0.37%) and 0% in 2017 (FTD 0%, RD 0%). Since the difference in anti-HBc prevalence in 2004/2013 and 2013/2017 among FTD is not statistically significant, it is reasonable to assume that exposure to HBV is continuously low in this age group. Croatia registers relatively few FTDs (approx. 10%), and they are recruited in younger age groups. Hence, 81% of FTDs tested for anti-HBc were younger than 29 in 2017, which would imply that they had received a mandatory vaccination against Hepatitis B virus.

Serological HBV profiles of anti-HBc positives did not change significantly over the period studied. The rate of anti-HBc-only among the anti-HBc positives was 11.8% in 2004, 9.6% in 2013 and 16.4% in 2017. Similar prevalence were reported in studies conducted in England (11.8%) [10] and Italy among FTD (10.4%) [11]. However, a statistically significant difference was found in the frequency of anti-HBc-only among the anti-HBc positives in blood donors younger than 30 years, mainly related to a general reduction of HBV prevalence in our donor population.

Anti-HBs positive rates among anti-HBc positive BDs were very similar during all three periods (80.56% in 2004, 87.57% in 2013 and 82.09% in 2017). The rates of BDs who were anti-HBs negative or having anti-HBs titer less than 100 IU/L (45.9% in 2004, 44.9% in 2013 and 47.7% in 2017) or less than 200 IU/L (52.5%, 56.8% and 59.6%, respectively) were very stable over the whole period studied.

In 2004, the rate of anti-HBe positives among anti-HBc positives was 37.1% and 33.2% in 2013 indicating stable serological profile in HBsAg-negative and anti-HBc positive BDs. There was only one registered case of an anti-HBc IgM grey-zone result in 2004 and no case of HBe antigenemia.

HBV DNA or OBI was not detected in 2004 among 47 anti-HBc-only positives tested with an assay sensitivity of 6 and 54 IU/mL. The use of highly sensitive ID-NAT assays also failed to detect HBV DNA in 12,408 BDs in 2013 and 2017.

A simulation of introducing anti-HBc test in testing of BD in Croatia, based on anti-HBc prevalence in 2017 (1.32%; 95%CI 1.04%–1.67%) and 95,000 blood donors per year (18,000 FTDs and 77,000 RDs) and the annual average number of donations by repeat donors of 2.1 would lead to the loss of: a) 988–1587 blood donors in case of universal testing with elimination of all anti-HBc positives, which would correspond to a loss of 1869–2999 blood donations a year, b) 472–758 blood donors in case of universal testing with elimination of all anti-HBs positives with a titer of less than 100 IU/L, which would correspond to a loss of 893–1434 blood donations a year, c) 589–947 blood donors in case of universal testing with elimination of all anti-HBs positives with a titer of less than 200 IU/L, which would correspond to a loss of 1115–1790 blood donations a year, and d) 11–326 blood donors in case of testing of first-time blood donors only.

However, the present study did not evidence the presence of OBI infection among anti-HBc positives. In the first year of testing blood donors in Croatia with ID-NAT tests, the frequency of OBI was reported at 1:7031, whereas after the 3-years period, it was reported at 1:10,900 donations [12], followed by a further significant decrease: 1:98,494 in 2016, 1:28,495 in 2017 and 1:195,815 in 2018. The analysis of 23 OBI donor archive samples showed the consistency of anti-HBc positive results (100%), as opposed to ID-NAT (63%) and ID-NAT reproducibility (50%), as expected for samples with a low HBV DNA viral load [13]. Those data support the importance of anti-HBc testing in identifying OBI donors.

Recently, anti-HBc prevalence of < 2% [14] has been proposed as a possible limit at which HBV safety is increased without critical impact on donor deferral. High anti-HBc prevalence does not always imply high OBI frequency. Namely, the rate of HBV DNA positives among anti-HBc positives varies in the range of 0–100% [15]. Another important factor is the contribution of anti-HBc tests to the reduction of HBV blood transmission risk, that can best be assessed by analysing results of look-back studies conducted in OBI donors and blood product recipients. Transmission rates vary and depend mostly on the recipient's availability for monitoring the infection within an adequate time-frame. Look-back procedures conducted for OBI in the Netherlands, Japan and Australia have established low HBV transmission rates from OBI donors of 5%, 3.5% and 0.2–3.2% [16–18]. A much higher transmission rate (28%) associated with plasma viral load in blood components and presence of anti-HBs in both donors and recipients was reported in a multi-centric European look-back study that included data from Croatia [19]. Differences in OBI frequency are attributed to genomic and epigenomic factors affecting DNA, viral and host's' hepatocytes. Robust evidence for genotype-dependent frequency of OBI are still missing but this might be another important factor in assessing the usefulness of anti-HBc testing in a given geographical area [20].

5. Conclusion

During the period 2004–2017, a significant 4-times decrease in anti-HBc prevalence in Croatian blood donors from 5.24% to 1.32% was observed. No OBI infection among anti-HBc positive BDs was detected in this study. Due to the high consistency of anti-HBc in Croatian OBI BDs, a strategy of universal testing of blood donors for this marker in addition to existing HBsAg and ID-NAT screening in Croatia would represent an additional measure to prevent HBV transmission by blood and blood components. By deferring all anti-HBc positive donors, at least 1.32% of blood donors should be substituted. Testing of FTDs only would be a less efficient method of risk reduction because: a) in Croatia OBI donors have been identified only in RDs (age group 43–67, M 58 years), b) FTDs mostly consist of young people who have already been vaccinated against HBV virus, and c) results from the most recent study have identified no anti-HBc positives among donors younger than 29 years of age. The decision to include mandatory anti-HBc testing in the existing BD screening strategy needs to be supported by further cost-benefit analyses.

Disclosure of interest

The authors declare that they have no competing interest.

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